

COMPARISON OF GLUCOCORTICOID CYTOPLASMIC RECEPTORS FROM LIVER, ZAJDELA HEPATOMA AND HTC CELLS

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1. Introduction

Glucocorticoids induce the synthesis of tyrosine amino transferase (EC 2.6.1.5) (TAT) in rat liver cells [1,2] and in hepatoma tissue culture cells (HTC) [3]. Specific glucocorticoid receptors have been described in these cells [4–6]. Zajdela hepatoma cells, which derive from rat liver, did not show any increase in TAT activity in response to glucocorticoid and especially to dexamethasone; furthermore, the number of cytoplasmic receptors was in these cells very much reduced (unpublished results).

In the present study we give further evidence demonstrating the impairment of receptor activity in Zajdela cells. We have compared the binding in three types of cells and the results suggest the existence of a regulatory mechanism in the binding of steroid hormones in target cells.

2. Material and methods

2.1. Preparation of cell extracts

Zajdela hepatoma cells were grown in vivo as described by Zajdela [7]. The cells were harvested and homogenized as previously described [8]. HTC cells were grown at 37°C in suspension cultures in SWIM's 77 medium supplemented with 10% calf serum [9].

After harvesting, the cells were treated in the same way than the Zajdela cells. Livers from male Wistar rats were excised after having been perfused in situ with 2 × 20 ml of ice-cold 0.9% NaCl and 20 ml of ice-cold homogenization buffer. Cytosol from rat liver and hepatoma cells were prepared following the procedure described by Beato et al. [5].

2.2. Steroid binding

In vitro binding of steroids to cytoplasmic proteins was performed either at 0°C or at 20°C in incubation buffer: 0.05 M Tris–HCl (pH 7.55), 5 mM MgCl₂, 1 mM EDTA, 1 mM mercaptoethanol with [³H]-dexamethasone (27 Ci/mmol) (Amersham Radio-

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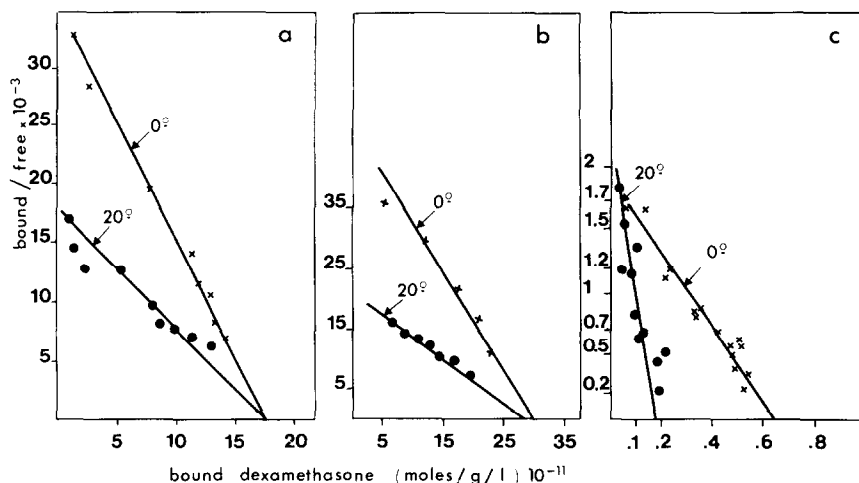


Fig. 1. Scatchard plots of the dexamethasone cytosol binding proteins. Cytosol proteins from: (a) rat liver (7.8 mg/ml), (b) HTC (2 mg/ml), (c) Zajdela (98.4 mg/ml) were incubated for 120 min at 0°C, or 20 min at 20°C, with various concentrations of [³H]-dexamethasone in the absence and in the presence of unlabelled 10⁻⁶ M dexamethasone. After incubation, the bound radioactivity was measured by Sephadex G75 gel filtration. The results of the specifically bound dexamethasone were plotted according to Scatchard.

chemical Center), either alone or in the presence of competing non radioactive dexamethasone in order to correct for non specific binding, the final volume was 0.5 ml. After incubation, the bound radioactivity was assayed either by the charcoal method according to Milgrom et al. [10], or by the gel filtration technique as previously described [11].

3. Results

3.1. Interaction of [³H]dexamethasone with the cytosol proteins at 0°C and 20°C

The amount of bound labelled dexamethasone to cytosol proteins from the three types of cells was determined as a function of the free steroid concentration present in the incubation medium, at 0°C and 20°C. Specifically bound hormone was determined after equilibrium had been reached in each condition. The data was plotted according to Scatchard [12] (fig. 1). For each experiment, the Scatchard plot was linear and it was analyzed as representing an homogeneous class of binding sites. Several independent experiments were performed with different preparations of Zajdela cytosol, the protein concentration ranged from 4 to 10 mg per ml.

The apparent association constant of HTC and liver cytosols at 0°C were $1.6 \times 10^8 \text{ M}^{-1}$ and $2 \times 10^8 \text{ M}^{-1}$ respectively. These values were similar to those given by Baxter and Tomkins [4] and by Beato et al. [13]. The constant of Zajdela cell cytosol was $2.5 \times 10^8 \text{ M}^{-1}$ at 0°C; it was higher at 20°C ($1.3 \times 10^9 \text{ M}^{-1}$) as compared to the results obtained from the cytosol of the HTC and liver cells ($1-0.7 \times 10^8 \text{ M}^{-1}$). Furthermore, the binding activity was the same at 0°C and 20°C for both liver and HTC cell cytosols but there was a diminution of 64% in the concentration of the binding sites at 20°C for the Zajdela cells.

The kinetics of association and dissociation of dexamethasone confirmed these results. The association of dexamethasone to Zajdela cells cytosol was completed in 90–120 min at 0°C and in 20 min at 20°C, using $2 \cdot 10^{-8} \text{ M}$ [³H]dexamethasone. The association followed second order kinetics (fig. 2a). The association constants for liver and Zajdela cell cytosol were respectively: at 0°C: 5.4 and $7.5 \times 10^5 \text{ M}^{-1} \text{ min}^{-1}$; at 20°C: 4.3 and $7.3 \times 10^6 \text{ M}^{-1} \text{ min}^{-1}$. The dissociation of dexamethasone from Zajdela cells and liver cytosols followed first order kinetics. At 0°C, the rate of dissociation was identical in both cytosols and the $t_{1/2}$ was 215 min. At 20°C, a two step graph was observed. Only 15% of the Zajdela complexes

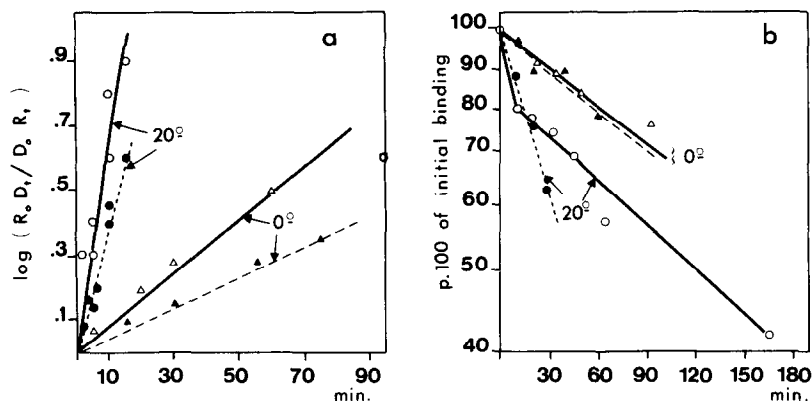


Fig. 2. Kinetics of dexamethasone association and dissociation with the cytoplasmic receptors from Zajdela and liver cells. (a) Rate of association: at zero time, 2.10^{-8} M [3 H]dexamethasone was added to cytoplasmic extracts (final concentrations: 10 mg/ml for liver cytosol, and 8.4 mg/ml for Zajdela cytosol). At the indicated times specifically bound dexamethasone was determined by the Sephadex procedure. Since the reaction follows second order kinetics, the rate constant of association, k_1 was calculated from the equation:

$$t = \frac{2.303}{k_1 (D_o - R_o)} \text{ or } \log_{10} \frac{R_o D_t}{D_o R_t}$$

where the initial concentration of dexamethasone, D_o , was 2.10^{-8} M; the concentration of dexamethasone binding sites, R_o , was $3.6 \cdot 10^{-10}$ M in liver cytosol and $0.6 \cdot 10^{-10}$ M in Zajdela cytosol. R_t is the concentration of free binding sites and D_t the concentration of free steroid at the given time. (b) Rate of dissociation: aliquots of the same cytosol preparation were incubated for 15 hr at 0°C. At the end of the incubation, the free steroid was removed by the charcoal procedure to determine the zero time. Dissociation was performed either at 0°C or at 20°C. At various times, the specifically bound dexamethasone was determined. The data were calculated as per cent of initial binding. Liver: (---); Zajdela: (—).

behaved similarly to the liver complexes. The major part of Zajdela complexes had a much slower rate of dissociation than the liver, giving $t_{1/2}$ of 45 min and 210 min respectively (fig. 2b).

The ratio of the rate constants of association and dissociation was used to calculate the equilibrium association constant. The results were in good agreement with those obtained from Scatchard plots (table 1).

3.2. Effects of mixing cytosols on the binding activity

The number of hormone binding sites was calculated from the Scatchard plots: 850 have been found for Zajdela cells, 8000 for liver cells and 16 000 for HTC cells. The difference in the binding activity for each cytosol could simply be due to the difference in apparent binding sites. An alternative hypothesis might be that the binding activity could be affected by the

Table 1
Constants for dexamethasone binding to cytoplasmic receptors

	Liver cytosol		Zajdela cytosol	
	(0°C)	(20°C)	(0°C)	(20°C)
k_1 ($M^{-1} \text{ min}^{-1}$)	5.4×10^5	4.3×10^6	7.5×10^5	7.35×10^6
k_1 (min^{-1})	3.7×10^{-3}	1.6×10^{-2}	3.7×10^{-3}	4.2×10^{-3}
k_1/K_d (M^{-1})	1.46×10^8	2.7×10^8	2×10^8	1.8×10^9
k_A (M^{-1})	2×10^8	1×10^8	2.5×10^8	1.3×10^9

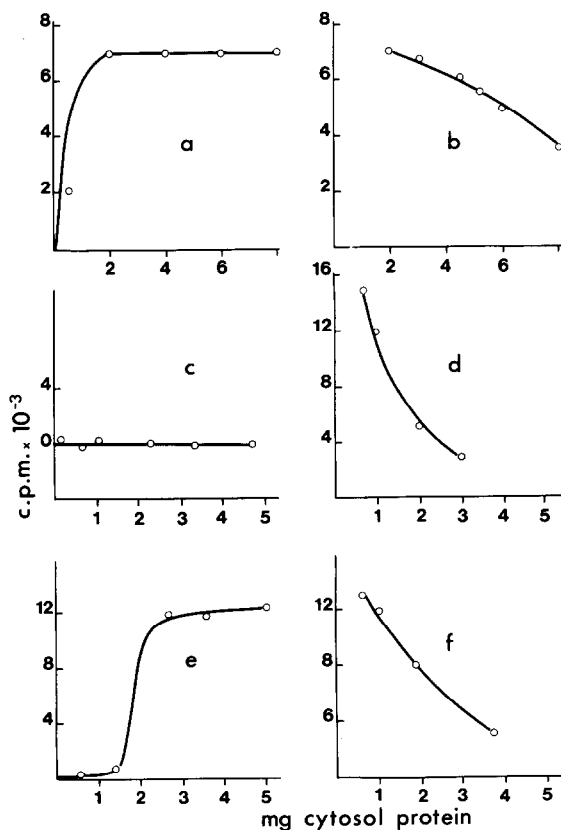


Fig. 3. Cross experiments. Increasing amounts of cytoplasmic protein from one type of cells were incubated in the presence of a constant amount of cytoplasmic proteins from another type of cell and with 2×10^{-8} [^3H]dexamethasone. The curves represent the bound radioactivity after subtraction of the binding by each cytoplasmic receptor incubated separately. (a) liver cytosol added to Zajdela cytosol (2.2 mg). (b) Zajdela cytosol added to liver cytosol (2.4 mg). (c) Zajdela cytosol added to HTC cell cytosol (1.8 mg). (d) HTC cell cytosol added to Zajdela cytosol (2.2 mg). (e) Liver cytosol added to HTC cell cytosol (1.8 mg). (f) HTC cell cytosol added to liver cytosol (2.4 mg).

presence or absence of various modulators.

In order to test this hypothesis we added liver cytosol to Zajdela or to HTC cytosol, HTC cytosol to Zajdela or to HTC cytosol, HTC cytosol to Zajdela or liver cytosol and Zajdela cytosol to HTC or liver cytosol (fig. 3). The experiments were performed in a range of proteins concentration in which the binding to each cytosol was linear. The following results were obtained: the addition of liver cytosol to Zajdela

cytosol increased the binding of dexamethasone. Conversely Zajdela cytosol inhibited the steroid binding when added to liver cytosol. The Zajdela cytosol had no effect on the binding by HTC cytosol, but the HTC cytosol, when added to Zajdela or liver cytosol, inhibited the binding. The liver cytosol increased the binding by HTC cytosol.

4. Discussion

Dexamethasone has been shown to induce TAT activity in liver and in HTC cells [1–3]. But we found that it failed to induce this activity in Zajdela cells. We have, therefore, investigated the characteristics of dexamethasone binding. Whereas the kinetic constants were found to be identical in liver and HTC cells, Zajdela cytosol binding was different in several ways. First, the number of binding sites was only 850 at 0°C . Second, we have observed that the Zajdela binding sites were decreased by 64% when studied at 20°C , compared to liver or HTC cells. Third, at 20°C , the K_A of Zajdela cell cytosol was higher than at 0°C ; this could partly be explained by a much lower rate of dissociation of the steroid receptor complex.

Cross-experiments between the cytosols of the three types of cells suggested a regulatory mechanism of hormone binding which is impaired in the liver derived cells. Zajdela cytosol inhibited liver cytosol dexamethasone binding. HTC cytosol had the same effect on liver and Zajdela cytosols, whereas Zajdela cytosol had no effect on HTC cytosol. To explain these observations, we would like to suggest the following hypothesis (fig. 4): in rat liver cells an equi-

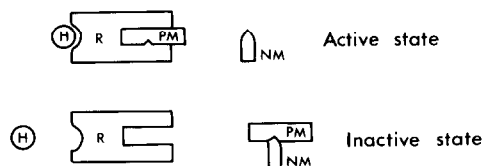


Fig. 4. Model of regulation of hormone binding to the cytosol receptor. The receptor molecule (R) contains two binding sites: a binding site for the hormone (H) and a binding site for the positive modulator (PM). The positive modulator can bind to a negative modulator (NM) which prevents its binding to the receptor.

brium exists between three components: (a) the receptor, (b) a positive modulator (PM) and (c) a negative modulator (NM). The binding of a positive modulator to the receptor molecule may induce a change of conformation of the molecule: it may be able to bind to, and later on, to dissociate the steroid. Under physiological conditions, the translocation into the nucleus would occur before the dissociation step. A negative modulator (NM) when associated to free PM could prevent the binding of PM to the receptor. Therefore, in cytosol, the receptor activity may partly be dependent on PM and PM–NM equilibrium.

In rat liver cells, the three types of molecules may be present in equilibrium, but free PM would be in excess. In HTC cells, the binding of NM to PM may be impaired, which allows a large increase of steroid binding resulting in an excess of free NM. In Zajdela cells, the number of PM would be reduced, which explains the low level of binding sites and also the reduction of the dissociation rate.

If this hypothesis is correct, we can assume that the hormone-receptor–PM complex is translocated into the nucleus. Within the nucleus, the dissociation of this complex begins with the release of the PM which becomes available for use again in the cytosol.

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